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Short communication

# Preliminary investigation of the application of on-line membrane extraction of trifluoroacetic acid as an aid to improvement of negative ion electrospray mass spectrometry data

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**Abstract**

We have recently investigated the biodegradation of a number of acidic aromatic compounds that give excellent chromatography using trifluoroacetic acid (TFA) based HPLC methods. Unfortunately HPLC methods using TFA are not usually compatible with detection by negative ion mass spectrometry as TFA suppresses ionisation of the analyte during the electrospray process. We present a preliminary investigation of the use of an anion-exchange micro-membrane suppressor to remove TFA on-line post column with the aim of improvement of mass spectral data using an aromatic acid as an example. Thus LC–MS using a TFA based HPLC method with negative ion mass spectral detection is shown to be possible with good sensitivity. 

*Keywords:* Electrospray ionization; Liquid chromatography–mass spectrometry; Interfaces, LC–MS; Trifluoroacetic acid

## 1. Introduction

Many of the projects investigated by the Environmental Research Group at SmithKline Beecham involve environmental fate and effect studies of a diverse range of chemical compounds associated with drug production. These include degradation (photo, chemical or biological) studies of given compounds often requiring the use of LC–MS to identify the products formed [1].

HPLC methods using trifluoroacetic acid (TFA) are not usually compatible with detection by negative ion electrospray (ESI) mass spectrometry as TFA suppresses ionisation during the electrospray process. The incompatibility of TFA based HPLC methods linked to mass spectrometry is mainly due to the high conductivity and surface tension of highly aqueous TFA solutions. Also for some basic compounds ion pairing causes significant signal reduction. In some cases the surface tension of TFA contributes to distortion of the aerosol produced in the electrospray and suppression of mass spectral signal is observed [2]. The net result of the properties of the solutions caused by the presence of TFA is a reduction in sensitivity for positive ion mass spectral detection and typically complete suppression of the analyte signal for negative ion mass spectral detection.

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To investigate 4-fluorocinnamic acid, using LC–MS, further chromatography method development was necessary to methods utilising eluents compatible with mass spectrometry detection [3] for instance ammonium acetate or ammonium carbonate. This is time consuming, moreover it would be an advantage to be able to transfer the method directly to the mass spectrometer for identification of unknowns, so as to retain chromatographic integrity.

Some solutions have been developed to counteract the mass spectral signal suppression effects of TFA containing mobile phases used for LC–ESI-MS. These include the post column addition of materials designed to counter the surface tension effects of TFA [3] or the redevelopment of LC–MS methods to alternative eluents [4]. Additionally, membranes have been used to extract materials from eluents before or after the chromatography. This type of technique was used as a method for sample clean up or desalting prior to LC–MS (for examples see [5,6], for a review see [7]). The use of an anion micro-membrane suppressor (AMMS) to reduce the background signal and hence improve signal-to-noise ratio is commonly used in suppressed ion chromatography with conductivity detection [8]. It has also previously been shown that it is possible to link suppressed ion chromatography to mass spectrometry [9] and that eluents not usually compatible with mass spectrometry such as sodium hydroxide can be utilised [10,11]. A post column removal of non-volatile buffers using a suppressor has also recently been reported [12] in a study on LC–MS of pesticides. We applied the concept of eluent suppression to reversed-phase HPLC linked to ESI-MS and using AMMS, attempted to remove TFA from the mobile phase post column. The experiment was designed so as to remove TFA from HPLC eluent containing water–acetonitrile–TFA, so as the analyte entered the mass spectrometer in a stream of water–acetonitrile with a reduced amount of TFA present.

## 2. Experimental

An HP1100 liquid chromatography system (Agilent Technologies, Manchester, UK) was coupled to a Quattro LC (Micromass, Wythenshawe, UK) triple quadrupole mass spectrometer operated in negative ion electrospray ionisation.

The anion-exchange micro-membrane suppressor (AMMS, Dionex, Leeds, UK) was used to remove trifluoroacetate ions, operating in chemical suppression mode. The regenerant, tetrabutylammonium hydroxide (Sigma–Aldrich, UK) (7 g $l^{-1}$, 30 m*M*) was pumped at a flow-rate of 0.2 ml $min^{-1}$ using an Applied Biosystems 400 solvent delivery system, (Applied Biosystems, Manchester, UK). This was used for accurate control of the regenarent flow. The AMMS was placed in-line between the column and the diode-array detector of the HPLC system.

A schematic of the mode of action of the AMMS is shown in Fig. 1. The AMMS is set up so that a flow of tetra butyl ammonium hydroxide exists on one side of the membrane and eluent on the other. Hydroxide ions cross the membrane to an area containing HPLC mobile phase, to undergo neutralization with hydronium ions in the eluent. To counter the charge inbalence, TFA anions cross the membrane in the opposite direction into the waste stream. The net result is the eluent leaving the AMMS contains a lower concentration of TFA. The concentration of TFA may be controlled by the flow-rates of the regenarent and the eluent flow.

HPLC was performed using a Symmetry $C_{18}$, 5 μm, 150×3.9 mm column (Waters, Watford, UK). The elution was isocratic using water–acetonitrile (60:40, v/v) with 0.1% (v/v) TFA; all chemicals used to prepare eluents were obtained from BDH, Poole, UK. The flow-rate was set to 1.0 ml $min^{-1}$ and the column oven to 40°C. UV detection was at 254 nm. An injection volume of 25 μl was used.

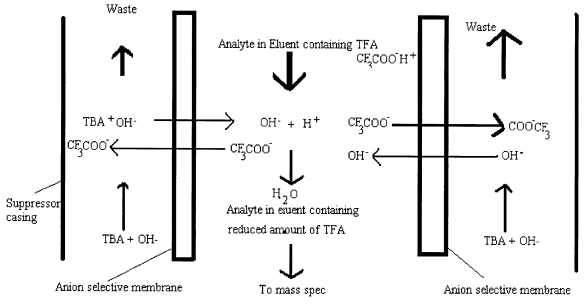
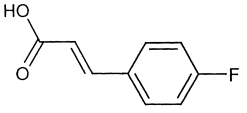



Fig. 1. Schematic of mode of operation of anion micro-membrane suppressor.

Eluent exiting HPLC at 1.0 ml $min^{-1}$ was split to approximately 200 μl $min^{-1}$ prior to entering the ion source of the mass spectrometer. Experiments were carried out with a capillary voltage of 3.0 kV, a cone voltage of 25 V, a desolvation temperature of 300°C and a source temperature of 120°C. The nitrogen desolvation and nebuliser gas flow-rates were set to 700 l $h^{-1}$ and 90 l $h^{-1}$ respectively. Data were acquired over a mass range of 50 to 400 u using a cycle time of 1.1 s.

The sample analyte used was 4-Fluorocinnamic acid (4-FCA, structure **I**, nominal molecular mass 166) at a level of 0.6 mg $ml^{-1}$ (supplied by Aldrich, Gillingham, UK).




**I**

The pH of eluent entering and leaving the AMMS was measured using a pH meter (Hanna Instruments, Leighton Buzzard, UK) calibrated with buffer solutions of pH 4.01 and pH 7.00. The measurement of pH in solutions containing organic solvent are probably shifted slightly, however these data were used for comparison purposes only, i.e. the effect of the solvent was assumed to be negligible in this case.

## 3. Results and discussion

A solution of 0.1% TFA in water has a pH of 2.0. As TFA is removed from the eluent the pH of the eluent rises. This is dependant on the flow-rates of the regenerant and of the eluents. As the analyte of interest is a weak acid, if too much TFA is removed the pH will rise too far so the analyte would ionise and therefore be extracted by the AMMS. Therefore the flow-rate of eluent and regenerant are critical. The pH of the eluent entering the AMMS was 2.0 and leaving the AMMS was 4.0.

Fig. 2 illustrates the lower total ion current (TIC) background signal when the AMMS is placed in-line when using the HPLC and regenarent flow conditions mentioned above.

Fig. 3 shows (a) the UV trace and (b) the TIC trace for unsuppressed LC–MS of 4-fluorocinnamic acid and (c) the UV trace and (d) the TIC trace for suppressed LC–MS. Although Fig. 3a shows a peak at 3.3 min, no peak relating to 4-FCA was observed in Fig. 3b. Conversely Fig. 3c shows a peak detected by UV at 3.3 min but the TIC for suppressed LC–MS (Fig. 3d) shows a discrete peak and a marked improvement in the mass spectral data in terms of sensitivity. (Note that both sets of MS data were manipulated by subtracting all background ions due to TFA (i.e., $[TFA\text{-}H]^- = m/z$ 113, 114, 115 $[2TFA\text{-}H]^- = 227$, 228 and 229 and $[2TFA + Na\text{-}2H]^- =$ 249, 250, 251). A peak due to 4-FCA may actually be observed in the non background-subtracted mass spectrum for the run using the AMMS. We were

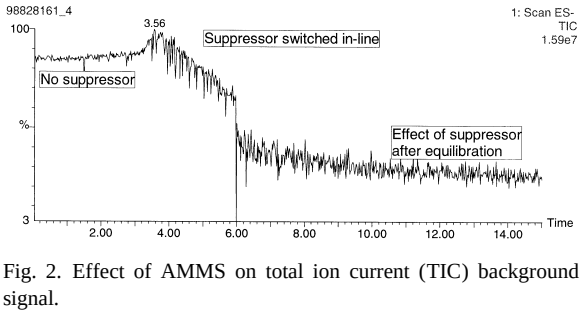



Fig. 2. Effect of AMMS on total ion current (TIC) background signal.

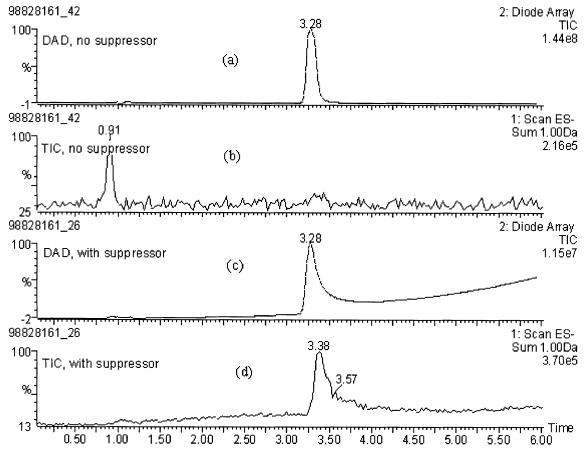



Fig. 3. UV and TFA related ions subtracted total ion current chromatograms for the LC–MS measurement of FCA. (a) UV trace unsuppressed (b) TIC unsuppressed (background selectively subtracted) (c) UV suppressed (d) TIC suppressed (background selectively subtracted).

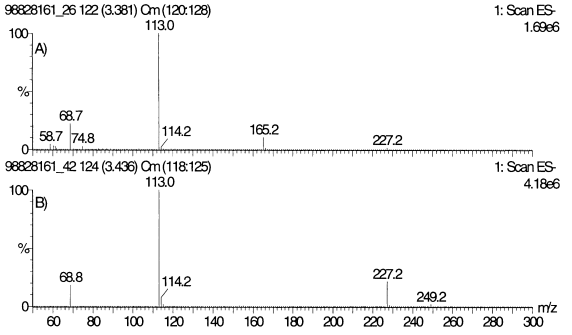



Fig. 4. Non-background subtracted LC–MS spectrum for FCA. (a) suppressed, (b) unsuppressed. An $(M\text{-}H)^-$ ion is observed at $m/z$ 165 when using the AMMS.

unable to obtain mass spectral data on the peak at 0.91 min in Fig. 3b; presumably this peak was due to the solvent front, exaggerated because of normalisation of chromatogram.

The sensitivity for the detection of FCA in negative ion electrospray is enhanced. Moreover, the response for FCA is increased by more than two orders of magnitude in absolute intensity ($1.7 \cdot 10^3$ without AMMS versus $2.5 \cdot 10^5$ with AMMS) and in area (area increased by a factor of 450). This shows that there is less suppression of ionisation of FCA by TFA when the AMMS is used. A small increase in noise in the mass spectral signal was noted, but from these data it is estimated that the use of the AMMS still enhances the signal-to-noise ratio by a factor of about 20. The AMMS appears to cause some band broadening but the quality of the mass spectra obtained are still good enough for structural elucidation.

Fig. 4 shows a comparison of the mass spectra taken from the peak detected by UV at 3.4 min with and without the AMMS in-line. Fig. 4a clearly shows an ion at $m/z$ 165 the $(M-H)^-$ for 4-FCA which is not visible in Fig. 4b acquired when the AMMS is not used.

## 4. Conclusion

We have shown the concept of improvement of mass spectrometry data using on-line membrane extraction of TFA post column with an AMMS. The removal of trifluoroacetate ions was found to increase the pH of the eluent stream post column (compared to pre-column) which aids the formation of anions.

For the example shown above, the membrane was used at a fraction of the total capacity so as not to suppress the analyte. The robustness of the system used in this way still needs to be proven. Optimisation regarding the flow-rate of the regenerant and its concentration should be investigated and a model for the movement of materials across the membrane constructed. Further work to investigate this is ongoing.

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